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# Enantioselective synthetic thalidomide receptors based upon DNA binding motifs

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A series of molecularly imprinted polymer (MIP) synthetic receptors selective for the sedative thalidomide (**5**) have been designed and synthesized based upon the functional monomer 9-(2′-methacryloyloxyethyl)adenine (**2**). $^1$H-NMR studies were used to establish the existence of DNA-like binding interactions between **2** and the template (**5**). A series of ethylene glycol dimethacrylate cross-linked copolymers was synthesized using either **2** or methacrylic acid, or a combination of these functional monomers. Zonal HPLC studies demonstrated enantioselectivity ($\alpha$ = 2.11) and ligand selectivity which could be attributed to the interaction of **2** with the imide moiety of **5**. Compound **2** provided a more significant contribution to the binding of **5** than methacrylic acid, though a combination of these two functional monomers resulted in improved enantioselectivity. Frontal chromatographic and batch binding studies confirmed the observed differences in affinity of the imprinted and reference polymers for the template.

## Introduction

Molecular imprinting[1,2] provides a relatively facile method for the preparation of synthetic polymers with recognition sites with antibody[3,4] or enzyme[5–7] like properties. The technique involves the use of reversible complexes (covalent or non-covalent) of functional monomers and a target structure, or template, which are polymerized in an excess of a crosslinking agent to produce rigid polymer networks. The template is removed to reveal complementary sites with selective recognition for the template, or related structures. The utility of the technique is to a large extent due to the range of chemical classes that are amenable to the process.

A growing number of functional monomer types have been studied in order to improve the ligand selectivity of molecularly imprinted polymer (MIP) systems. Reversible covalent interactions provide well defined complexes which in turn favour the generation of recognition sites of high fidelity. Unfortunately, the number applicable to use in molecular imprinting protocols is relatively limited, primarily due to ligand–template rebinding kinetics. The use of non-covalent interactions[8] for functional monomer–template recognition, while in many cases easier to use, introduces complicating factors due to the often transient nature inherent to these interactions.[9] This results in a continuum of complex types, including template–template interactions[10,11] which is reflected in the heterogeneity of the recognition sites present in the resultant polymers. Accordingly, spectroscopic,[12–14] molecular modeling[15,16] and combinatorial[17,18] methods have been used to identify suitable monomer systems, and to characterize their interaction with templates.

The use of covalent interactions in conjunction with non-covalent recognition has been demonstrated to be a viable compromise between the two techniques.[19] More recently the utility of multidentate functional monomers capable of stoichiometric interactions with templates has been demonstrated, *e.g.* amidine–carboxyl interactions.[20,21] Such polymers possess the advantages of covalent imprinting in conjunction with the versatility of the non-covalent interaction based strategy. New functional monomer types capable of simultaneous multiple interactions with a template species should aid in the development of novel polymer systems suitable for use with other classes of templates. A number of other multidentate systems have been presented utilizing various types of interaction, *e.g.* metal–ion coordination,[22–24] the hydrophobic effect[25–27] and hydrogen bonding motifs.[28,29]

The energetic advantage gained through the use of a functional monomer capable of simultaneous multiple non-covalent interactions, relative to multiple monomers engaging in single interactions, lies primarily in the reduction of the translational and rotational free energy penalty associated with formation of higher order complexes.[30,31] Such examples are abundant in biological systems, *e.g.* in the base-pairing present in the double helical structure of DNA and virus–cell surface interactions. The intramolecular bonding motifs underlying the structure of DNA have been employed in a number of biomimetic receptor systems designed for the selective recognition of nucleotide bases.[32]

Molecular imprinting has provided yet another approach for the fabrication of systems selective for nucleotide bases.[33–35] In these cases, the functional monomer methacrylic acid was used for electrostatic interaction based recognition of a series of nucleotide bases and derivatives. In the present study, the hydrogen bonding motif present in the nucleotide base adenine (**1**) has been utilized in the development of a functional monomer, 9-(2′-methacryloyloxyethyl)adenine (MAOAd, **2**),[36,37] for use in molecular imprinting protocols. It was anticipated that this monomer would have selectivity for structures containing complementary hydrogen bonding sites as present in DNA and RNA in the cases of thymine (**3**) and uracil (**4**), Fig. 1.

The controversial sedative thalidomide (**5**) can result in teratogenic effects such as phocomelia (short limbs) and amelia

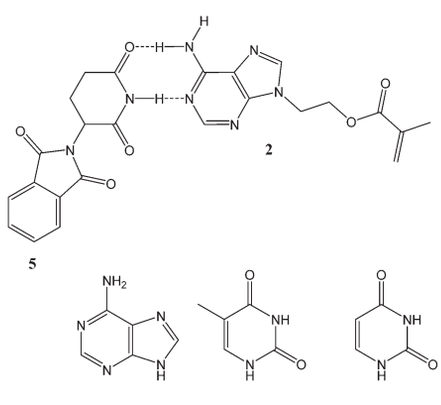


**Fig. 1** Anticipated Watson–Crick-like interaction between thalidomide (**5**) and MAOAd (**2**). Adenine (**1**), thymine (**3**) and uracil (**4**).

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(absence of limbs) if used during pregnancy.[38] Although banned in many countries, this substance is still used in the treatment of leprosy and has more recently returned to the clinic for use in the treatment of various forms of cancer and several HIV-related indications.[39] Thalidomide possesses an imide moiety analogous to that present in thymine and uracil and was perceived as a suitable target for a study using this monomer.

The suitability of **5** as a template for the development of molecularly imprinted polymers is further motivated by the possibility of developing recognition systems for use in clinical analysis, and even selective recognition of nucleotides and oligonucleotides, the subject of ongoing work in our laboratory. In addition, the fact that thalidomide contains a chiral centre, allows for more detailed studies of recognition site fidelity achieved using molecular imprinting protocols employing this monomer.

## Experimental

### Chemicals

(*R*,*S*)-Thalidomide (>98%), (*R*)-thalidomide, (*S*)-thalidomide (>98%) and $d_4$-acetic acid were all obtained from SIGMA, ethylene carbonate, sodium hydride (NaH, 95%), methacryloyl chloride, *N*-phthaloylglutamic anhydride (**7**, 98%), *N*-phenylphthalimide (**8**, 98%), *N*-methylphthalimide (**9**, 98%), and ethylene glycol dimethacrylate (EGDMA, 98%) were from Aldrich, adenine and methacrylic acid (MAA) were from MERCK, 2,2′-azobis(2-isobutyronitrile) (AIBN, 98%) was from Janssen Chimica. All solvents used were of analytical or HPLC grade.

### Synthesis of 9-(2′-hydroxyethyl)adenine (6)

A solution of **1** (4.96 g, 36.7 mmol), ethylene carbonate (3.30 g, 37.5 mmol) and a trace of NaOH in DMF (150 ml) was heated at reflux for 2 hours. After evaporation of the solvent under reduced pressure, the crude product was recrystallized from ethanol to give 4.68 g (71%) of **6** as colourless needles; mp 232–234 °C (Lit.[36] 238–239 °C); $\delta_H$(250 MHz; $D_6$-DMSO) 8.14 (1 H, s, ArH), 8.08 (1 H, s, ArH), 7.18 (2 H, s, $NH_2$), 5.00 (1 H, t, $J$ = 5.4 Hz, OH), 4.19 (2 H, t, $J$ = 5.5 Hz, $CH_2$), 3.75 (2 H, q, $J$ = 5.4 Hz, $CH_2$).

### Synthesis of 9-(2′-methacryloyloxyethyl)adenine (2)

NaH (0.5 g, 19.8 mmol) was added to a solution of **6** (3.18 g, 17.7 mmol) in DMF (250 ml) and was stirred at room temperature for 60 min. The reaction mixture was cooled to 0 °C and methacryloyl chloride (1.95 ml, 19.5 mmol) was added dropwise with stirring. After 2 h the solvent was evaporated under reduced pressure and the crude product was recrystallized from water to furnish **2** as a pale yellow solid (1.55 g, 35%); mp 193–196 °C (Lit.[37] 201–203 °C); $\nu_{max}$(KBr) 1720 $cm^{-1}$; $\delta_H$(250 MHz, $d_6$-DMSO) 8.15 (1 H, s, ArH), 8.14 (1 H, s, ArH), 7.20 (2 H, s, $NH_2$), 5.92 (1 H, s, CH), 5.62 (1 H, s, CH), 4.47 (4 H, s, 2 × $CH_2$), 1.77 (3 H, s, $CH_3$); $\delta_C$(62.5 MHz, $d_6$-DMSO) 166.1, 155.9, 152.4, 149.6, 140.9, 135.4, 126.1, 118.6, 62.6, 42.0, 17.8; *m*/*z* 247.1077, $C_{11}H_{13}N_5O_2$ requires 247.1069.

### NMR titration experiments

Spectra were recorded on a Bruker AC 250 MHz instrument at 295 K. Solutions of thalidomide (**5**) (5 mM in $CDCl_3$ or $CD_3CN$) were titrated with either a solution containing thalidomide (5 mM) and acetic acid-$d_4$ (1.75 M) or MAOAd (48 mM) and acetic acid-$d_4$ (143 mM). Apparent dissociation constants (app. $K_{diss}$) were calculated by non-linear regression using the software package Graph Pad Prism 3.02 (Graph Pad Software Inc., San Diego, CA, USA). NMR-data for Job-plot analysis were obtained using samples prepared in $CDCl_3$ containing different molar fractions of **5** and **2**, with a constant total concentration of 5 mM.[40]

### Polymer synthesis

In a typical polymer synthesis, (*S*)-thalidomide was mixed with functional monomers, crosslinker and porogen in a glass test tube, Table 1. The tube was placed in an ultrasonic water bath until clear solutions were obtained, then cooled on ice and the solutions were sparged with dry nitrogen for 5 minutes before the addition of AIBN. Polymerization was initiated by either UV-irradiation or heating. The resultant white polymeric monolith was manually ground and sieved through a 63 μm sieve, and fine particles were removed by repeated sedimentation from 400 ml acetone (10 × 20 min). Polymer particles (5 g) were slurry packed into an HPLC column and washed systematically following a protocol described by Karlsson *et al.* including both acidic and basic washing steps.[41] The polymers were emptied from the column, air dried and stored until use. A non-imprinted reference polymer was prepared as described above, though in the absence of thalidomide. The polymers were physically characterised using elemental analysis (performed by Mikrokemi AB, Uppsala, Sweden) and BET-adsorption analysis (Micrometrics ASAP 2400, samples were degassed at 100 °C for 24 h before analysis).

### Packing of HPLC columns

Washed polymers were suspended in chloroform/acetonitrile (85:15, v/v) in a slurry reservoir and packed into stainless steel HPLC columns (250 × 4.6 mm for zonal analysis and 50 × 3.1 mm for frontal analysis) at 290 bar using a single action reciprocating plunger pump (Haskel Engineering Supply Co., USA) with 400 ml acetone as the packing solvent.

### Zonal chromatography

Chromatographic evaluations were performed using an HPLC system comprised of a Series 200 LC pump (Perkin Elmer, USA), a 20 μl injection loop and a 785A programmable absorbance detector (Applied Biosystems). All analyses were performed in triplicate with a concentration of 194 μM of the analyte; (*S*)-thalidomide, (*R*)-thalidomide, *N*-phthaloylglutamic anhydride (**7**), *N*-phenylphthalimide (**8**) or *N*-methylphthalimide (**9**) in chloroform. Chloroform was used as the mobile phase (flow rate of 0.5 ml $min^{-1}$), and elution was monitored at 293 nm. The void volume ($V_0$) was determined by injections of cyclohexane, and the retention volumes ($V_R$) for analytes were assigned to the point on the peak corresponding to 50% of the peak area by manual integration using gravimetric analysis.

### Frontal chromatography

Frontal chromatography was performed on a Merck-Hitachi LaChrom HPLC system comprised of a series L-7100 pump, L-7200 autosampler, L-7455 diode array detector and a D-7000 interface. Sample solutions in chloroform (0.25, 0.4, 0,75 or 2.5 mM (*S*)- or (*R*)-thalidomide or 0.5% acetone) were continuously pumped through the columns at a flow rate of 0.5 ml $min^{-1}$, until a stable plateau was observed. All experiments were performed in triplicate.

### Equilibrium binding studies

Equilibrium binding studies were performed in screw cap glass vials with a polymer concentration of 15 mg $ml^{-1}$ and an (*S*)-thalidomide concentration ranging from 0.25 to 2500 μM in chloroform (1.0 ml). Duplicate samples were incubated on a rocking table for 16 h at 20 °C. 600 μl aliquots were then transferred to new HPLC vials *via* PTFE syringe filters and analysed on a silica column (50 mm × 4.6 mm id, silica 300, 5 μm) using the HPLC system described for zonal chromatography studies but fitted with a 200 μl injection loop. Elution was performed using chloroform containing acetic acid (1%, v/v), a flow rate of 1.5 ml $min^{-1}$ and was monitored at 243 nm. Standard solutions of (*S*)-thalidomide were similarly

**Table 1** Polymer compositions, methods for preparation and physical characterisation

| | P1 | | P2 | | P3 | | P4 | |
|---|---|---|---|---|---|---|---|---|
| | MIP | REF | MIP | REF | MIP | REF | MIP | REF |
| (*S*)-Thalidomide (mmol) | 0.19 | — | 0.19 | — | 0.19 | — | 0.19 | — |
| HOAc (mmol) | — | — | — | — | — | — | 3.48 | 3.48 |
| MAOAd (mmol) | — | — | — | — | 1.16 | 1.16 | 1.16 | 1.16 |
| MAA (mmol) | 4.66 | 4.66 | 4.66 | 4.66 | 3.48 | 3.48 | — | — |
| EGDMA (mmol) | 21.21 | 21.21 | 21.21 | 21.21 | 21.21 | 21.21 | 21.21 | 21.21 |
| AIBN (mmol) | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 | 0.59 |
| MeCN (ml) | 7.0 | 7.0 | — | — | — | — | — | — |
| $CHCl_3$ (ml) | — | — | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| M/T | 24/1 | 1/0 | 24/1 | 1/0 | 24/1 | 1/0 | 6/1[a] | 1/0 |
| UV at 365 nm, 22 °C | 1 h | 1 h | 1 h | 1 h | — | — | — | — |
| Heat, 70 °C | 16 h | 16 h | — | — | — | — | — | — |
| Heat, 55 °C | — | — | 16 h | 16 h | 17 h | 17 h | 17 h | 17 h |
| % C Found (calc.) | — | — | 57.8 (60.4) | 56.6 (60.4) | 58.3 (60.0) | 58.3 (60.0) | 57.9 (60.3) | 57.3 (60.3) |
| % H Found (calc.) | — | — | 7.2 (7.2) | 7.3 (7.2) | 7.1 (7.1) | 7.2 (7.1) | 7.1 (7.1) | 7.0 (7.1) |
| % N Found (calc.) | — | — | <0.3 (0.3) | <0.3 (0.3) | 1.4 (2.0) | 1.4 (2.0) | 2.1 (2.1) | 1.7 (2.1) |
| BET surface area ($m^2\ g^{-1}$) | 311 | 324 | 374 | 369 | 291 | 285 | 333 | 327 |
| Micropore area ($m^2\ g^{-1}$) | 128 | 131 | 167 | 174 | 122 | 111 | 160 | 160 |
| Micropore volume ($cm^3\ g^{-1}$) | 0.056 | 0.056 | 0.073 | 0.076 | 0.052 | 0.047 | 0.070 | 0.070 |
| Average pore diameter (Å) | 125.4 | 145.0 | 71.9 | 62.3 | 74.6 | 75.7 | 48.5 | 49.3 |

[a] This figure does not account for the use of HOAc as a monomer analogue, which renders an M/T ratio of 24/1.

treated in order to take into account eventual evaporation of solvent during incubation.

## Results and discussion

9-(2′-Methacryloyloxyethyl)adenine (**2**) was synthesized in two steps using a previously described procedure, Scheme 1.[36,37] The base catalysed nucleophilic addition of adenine to ethylene carbonate afforded 9-(2′-hydroxyethyl)adenine (**6**), which was treated with methacryloyl chloride in the presence of a slight excess of sodium hydride to furnish **2**.

A series of $^1$H-NMR studies was undertaken in order to establish the nature of interactions between thalidomide (**5**) and both the monomer (**2**) and methacrylic acid. Solutions of **5** in chloroform were titrated with acetic acid-$d_4$, an analogue of methacrylic acid,[13] which resulted in changes in the chemical shift of the phthalimide proton of **5**. No significant shifts of other proton resonances was observed, suggesting that functional monomer interaction is primarily with the phthalimide moiety. The saturation isotherm obtained allowed the calculation of an apparent dissociation constant (app. $K_{diss}$) of 0.132 ± 0.017 M. Attempted titrations of **5** with **2** proved fruitless due to the insolubilities of these substances. However titration of **5** with solutions of **2** containing acetic acid-$d_4$, showed not only downfield shifts of the phthalimide proton but also of the amine protons of **2**. An app. $K_{diss}$ based upon acetic acid concentration of 0.042 ± 0.003 M was obtained for the imide proton of **5**, and an app. $K_{diss}$ derived from the shift of the amine protons of **2** of 0.032 ± 0.007 M. The corresponding app. $K_{diss}$ values, based upon the concentration of **2**, were 0.014 ± 0.001 M and 0.011 ± 0.002 M, respectively. Although clear interpretation of the results of the double titration is difficult, it can be stated that the interaction of **2** with **5** is an order of magnitude stronger than with acetic acid. That conditions suitable for the solubilities of both **2** and **5**, and also the strength of their interaction, are apparently dependent upon the presence of acetic acid, suggests a role for a carboxylic acid functionality in the nature of the complex formed between **2** and **5**. Significantly weaker complexation was obtained in acetonitrile (data not shown).

Job's method of continuous variation[11,42,43] was used in order to determine the stoichiometry of solution complexes between both **5** and acetic acid, and **5** and **2**. The imide proton of **5** is engaged in a 1 : 1 complex with both acetic acid (data not shown) and with MAOAd. The amine of MAOAd is involved in a complex of 1 : 2 stoichiometry, Fig. 2. This result suggests a relationship analogous to that in a DNA triple helix, whereby the adenine engages in interaction with two other bases, or in this case base analogues. Collectively, the NMR studies provide support for the presence of effective interactions between **5** and both **2** and acetic acid.

Based upon the results of the NMR studies, a series of thalidomide molecularly imprinted polymers was synthesized consisting of four sets of polymers (imprinted and non-

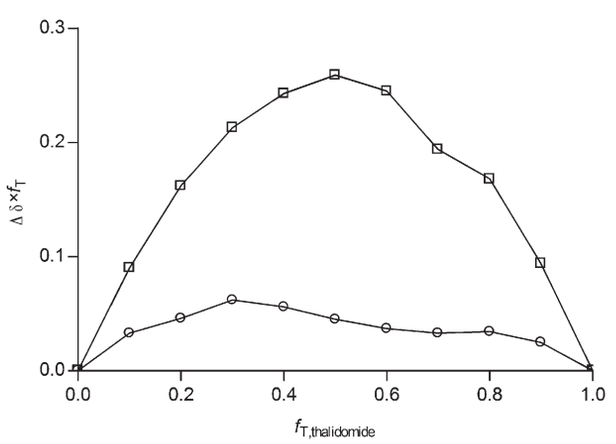
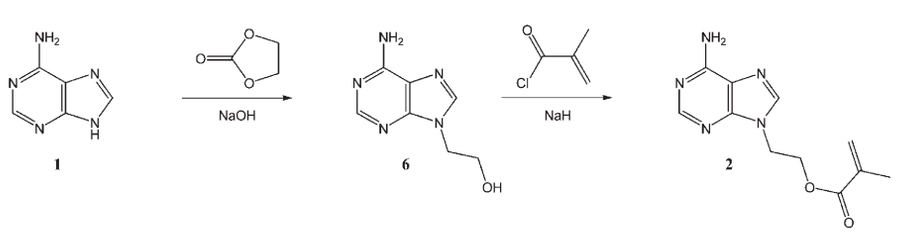



**Fig. 2** Job plots for the imide proton of **5** (□), and the amine protons of **2** (○). Total concentration: 5 mM in $CDCl_3$ (295 K).




**Scheme 1**

**Table 2** Polymer recognition characteristics from zonal chromatography performed in chloroform[a]

| | | (*S*)-**5** | (*R*)-**5** | **7** | **8** | **9** |
|---|---|---|---|---|---|---|
| P1 | $k'_{MIP}$ | 0.587 ± 0.003 | 0.568 ± 0.002 | 0.327 ± 0.004 | 0.012 ± 0.002 | 0.030 ± 0.002 |
| | $k'_{REF}$ | 0.648 ± 0.003 | 0.649 ± 0.003 | 0.407 ± 0.002 | 0.030 ± 0.001 | 0.053 ± 0.002 |
| | $R$ | 1.00 | 0.96 | 0.88 | 0.44 | 0.65 |
| P2 | $k'_{MIP}$ | 2.411 ± 0.025 | 1.321 ± 0.020 | 0.624 ± 0.001 | 0.036 ± 0.001 | 0.055 ± 0.001 |
| | $k'_{REF}$ | 1.031 ± 0.002 | 1.042 ± 0.001 | 0.540 ± 0.000 | 0.025 ± 0.000 | 0.042 ± 0.001 |
| | $R$ | 1.00 | 0.54 | 0.49 | 0.62 | 0.56 |
| P3 | $k'_{MIP}$ | 5.526 ± 0.070 | 2.616 ± 0.023 | 0.880 ± 0.006 | 0.056 ± 0.001 | 0.078 ± 0.001 |
| | $k'_{REF}$ | 1.725 ± 0.001 | 1.726 ± 0.004 | 0.781 ± 0.007 | 0.053 ± 0.003 | 0.078 ± 0.005 |
| | $R$ | 1.00 | 0.47 | 0.35 | 0.33 | 0.31 |
| P4 | $k'_{MIP}$ | 2.853 ± 0.056 | 2.278 ± 0.007 | 0.879 ± 0.009 | 0.045 ± 0.002 | 0.056 ± 0.001 |
| | $k'_{REF}$ | 1.743 ± 0.006 | 1.770 ± 0.012 | 0.755 ± 0.006 | 0.056 ± 0.004 | 0.082 ± 0.006 |
| | $R$ | 1.00 | 0.79 | 0.71 | 0.49 | 0.42 |

[a]Capacity factors ($k'$) were calculated using $k' = (V_R - V_0)/V_0$ where $V_R$ represents the retention volume (ml) of the substance studied and $V_0$ represents the void volume (ml). Normalised retention factors ($R$) were calculated as described previously.[44]

imprinted) using different combinations of monomers and porogens, Table 1. **P1** and **P2** were synthesized using methacrylic acid as functional monomer, **P1** in acetonitrile and **P2** in chloroform. **P3**, synthesized in chloroform, employed a combination of the two functional monomers **2** and MAA. The need for the use of MAA arose from solubility problems, in accordance with experience from the NMR studies. To investigate the role of the carboxyl group in conjunction with **2**, a set of polymers, **P4**, was included where MAA was substituted with acetic acid.

Photochemical initiation at 4 °C, followed by thermal annealing was employed for **P1** and **P2**. The solubility of the polymerisation mixture components, however, prohibited the use of an initial low temperature for the other polymer systems. Polymer monoliths were processed to obtain particles suitable for use in chromatographic studies, ≤63 μm diameter, and were washed under pressure using a series of pH and organic/aqueous solvent washing steps.[41] The observed gas accessible surface areas were somewhat higher than often observed for MAA–EGDMA polymers prepared in chloroform. We attribute this to the lower level of crosslinking (higher proportion of functional monomer) used in this system.

After packing into HPLC-columns, the retention characteristics of (*R*)- and (*S*)-thalidomide were examined by single injections, together with those of a number of structural analogues, Fig. 3 and Table 2. Chloroform was selected as the mobile phase on account of the fact that, based upon NMR data, it supports stronger complexation of the template by the functional monomer(s)/analogues than acetonitrile. The imprinted polymers prepared using methacrylic acid as functional monomer and acetonitrile as porogen, **P1**, demonstrated evidence of weak ligand selectivity and very poor enantioselectivity. Moreover, retention volumes were lower than those for the corresponding non-imprinted reference polymer, and the general poor recognition resulted in this polymer not being selected as a candidate for further experiments. In the case of **P2**, prepared as for **P1**, though with chloroform as porogen, the retention volumes on both imprinted and non-imprinted polymers were significantly larger than observed for **P1**, in particular for the template (**5**), and more distinct enantioselectivity was observed ($\alpha = 1.82$), as reflected in the normalised retention factors ($R$).

The combination of **2** with MAA in **P3** led to an enhancement of enantioselectivity ($\alpha = 2.11$). The improvement in enantioselectivity afforded by the incorporation of **2**, relative to **P2**, corresponds to a difference in free energy of enantioselective binding of 2.9 kJ mol$^{-1}$, at 295 K. The two structural analogues *N*-phenyl- (**8**) and *N*-methylphthalimide (**9**), each lacking the dioxopiperidinyl moiety, show essentially no retention. This result implicates the significance of this structural feature for recognition of the template. Importantly, substitution of the NH containing imide of **5** with an anhydride functionality (**7**) results in an intermediate retention volume, corresponding to a decrease in affinity for the polymer relative to that of the template. This reflects the loss of the capacity of the ligand to donate a hydrogen bond to the polymer. This particular interaction was demonstrated in the NMR studies described above to be of significance for interaction between the template and the nucleotide base-derived functional monomer. Collectively, these results provide support for the molecular imprinting central dogma, namely that the ligand selectivity of molecularly imprintined polymers is a consequence of the tempate-functional monomer interactions present in the pre-polymerisation mixture.

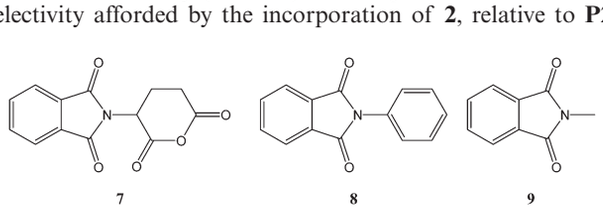


**Fig. 3** Structures of thalidomide analogues.

The enantio- and ligand selectivities of **P3** are further illustrated by the results obtained by co-injection of (*R*)-**5**, (*S*)-**5**, **7**, **8**, **9** and a void marker, cyclohexane, onto both **P3**, and its corresponding non-imprinted polymer, Fig. 4. On both polymers, **8** and **9** co-eluted with the void. **7**, however, showed an intermediate retention profile, with some peak width and tailing in the case of the MIP. The asymmetric peak broadening is possibly due to the racemic nature of the analyte, whereby one enantiomer is more strongly bound. Again, the significantly longer retention of the template, (*S*)-**5**, than its optical antipode on the imprinted polymer, together with the lack of enantioselectivity observed on the corresponding reference polymer, provide additional support for the presence of sites selective for the template.

As noted previously, the solubilities of **2** and **5** proved problematic, prohibiting the synthesis of polymers based solely upon **2** as the functional monomer. Thus, in order to provide a clearer picture of the influence of MAOAd (**2**) on the resultant

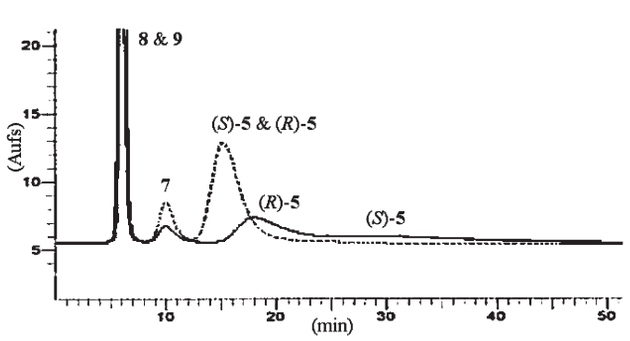



**Fig. 4** Injections (20 μl) of a solution containing (*S*)-**5**, (*R*)-**5**, **7**, **8** and **9** on **P3**, reference (each 19.4 μM, dashed line) and imprinted (9.7 μM, solid line) polymers.

recognition characteristics of the polymer, a set of polymers was synthesized where MAA was substituted with acetic acid in the polymerization, **P4**. The resultant polymers demonstrated a markedly lower enantioselectivity ($\alpha$ = 1.25, pure chloroform) than in the case of **P3**, primarily due to a decrease in the affinity of (*S*)-**5**. Relatively little influence on the retention characteristics of the analytes on the corresponding reference polymer was observed. This implies that the absence of carboxyl residues in **P4** mainly affects the nature of the high fidelity recognition sites. This is further supported by the retention of the anhydride, **7**, which was unaffected. Experiments were run using various quantities of acetic acid in the mobile phase in an attempt to see if **P4** could demonstrate selectivity for the complex of **5** and acetic acid, as used in the imprinting process. These studies showed a reduction in capacity factors and enantioselectivity for both the template and its optical antipode in the presence of increasing concentrations of acetic acid. Collectively, the HPLC data indicate that MAOAd (**2**) affords the most significant contribution to the recognition of the template, though the inclusion of MAA results in improved enantioselectivity through the provision of additional interaction points in the resultant polymers. Moreover, comparison of the retention data shows that MAOAd is the key source of ligand–polymer recognition of analytes containing the dioxopiperidinyl moiety.

To further penetrate the recognition characteristics of the polymers, a series of frontal chromatographic[45] and batch binding analyses were performed. The frontal chromatographic studies provide an estimate of the number of sites available for ligand interaction with the polymer, 21.3 ± 1.2 μmol sites $g^{-1}$ (dry weight) for **5** with **P3**, as compared to 17.8 ± 1.2 μmol sites $g^{-1}$ for the corresponding non-imprinted reference polymer. With this system, the enantioselectivity observed in the zonal chromatography was not evident on account of the overload conditions inherent to frontal chromatographic studies. Batch (equilibrium) binding studies were used as an alternative approach, though limits in the sensitivity the HPLC-based assay (detection limit 0.25 μM), together with solubility problems, narrowed the operative window significantly. Nonetheless, within the accessible concentration range, 0.25 to 2500 μM, a significant difference in binding to the imprinted and reference polymers was evident. At a concentration of 2.5 μM of (*S*)-**5**, the difference in binding to the imprinted and reference polymers corresponds to 0.022 μmol $g^{-1}$ polymer. Together with the zonal HPLC data, these results support the selectivity of the imprinted polymer for the template.

In conclusion, in the present work we have described the use of a multidentate functional monomer for use in molecular imprinting protocols. The DNA-like hydrogen bonding motif provided by the functionalized adenine is capable of engaging in non-covalent interactions with the complementary dioxopiperidinyl moiety present in thalidomide (**5**). The multiple hydrogen bonding possible with this monomer, in combination with methacrylic acid, furnishes the polymer with improved enantioselectivity for (*S*)-thalidomide, as compared with polymers prepared using only MAA. These results highlight the potential for the use of this, or related, polymer systems for the development of polymers with selectivity for nucleotide-like recognition motifs. The possibility of using this approach for the preparation of synthetic recognition sites selective for nucleotides is the subject of ongoing work in our laboratory.

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